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ORIGINAL RESEARCH

Sociability in Fruit Flies: Genetic Variation, Heritability and Plasticity

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Abstract
Sociability, defined as individuals' propensity to participate in non-aggressive activities with conspecifics, is a fundamental feature of behavior in many animals including humans. However, we still have a limited knowledge of the mechanisms and evolutionary biology of sociability. To enhance our understanding, we developed a new protocol to quantify sociability in fruit flies (*Drosophila melanogaster*). In a series of experiments with 59 F1 hybrids derived from inbred lines, we documented, first, significant genetic variation in sociability in both males and females, with broad-sense heritabilities of 0.24 and 0.21 respectively. Second, we observed little genetic correlation in sociability between the sexes. Third, we found genetic variation in social plasticity among the hybrids, with a broad-sense heritability of ~0.24. That is, genotypes differed in the degree of sociability after experiencing the same relevant social experience. Our data pave the way for further research on the mechanisms that underlie sociability as well as its ecological and evolutionary consequences.

Keywords *Drosophila melanogaster* · Fruit flies · Genetic variation, heritability · Plasticity · Reaction norms · Sociability, social behavior

Introduction

Social behavior, broadly defined as the interactions between conspecifics, has been subjected to extensive research in a broad range of organisms from bacteria to humans (Allee 1931; Wilson 1975; Ward and Webster 2016). A key aspect of social behavior is sociability, defined as the tendency to engage in non-aggressive group activities. Examples include feeding or roosting together, and traveling in a group. Sociability varies widely among animal species, between distinct ecological settings within a given species and among individuals within a population. For example, an analysis of social behavior among over 2500 mammalian species revealed a robust pattern of evolutionary transition from the ancestral solitary condition, which occurs in 68% of the species, to social monogamy (9%) and then to group living (23%) (Lukas and Clutton-Brock 2013). Within some carnivore species such as red foxes (*Vulpes vulpes*) and grey wolves (*Canis lupus*), food abundance and distribution dramatically alters sociability (Macdonald 1983; Johnson et al. 2002). Finally, Cote and colleagues (Cote and Clobert 2007; Cote et al. 2012) documented individual variation in sociability in both lizards (*Lacerta vivipara*) and fish (*Gambusia affinis*).

There has recently been increased interest in using fruit flies (*Drosophila melanogaster*) as a model system for research on social behavior. Although fruit flies are traditionally classified as solitary insects, they actually show a variety of social behaviors including aggregation at food sources, which is actively modulated through pheromones (Bartelt et al. 1985; Wertheim et al. 2006; Lin et al. 2015), social synchronization of the circadian clock (Levine et al. 2002), reliance on social information gleaned from conspecifics (Sarin and Dukas 2009; Battesti et al. 2012), and the formation of social groups (Saltz 2011; Schneider et al. 2012; Simon et al. 2012; Anderson et al. 2016). While numerous taxa have been used successfully for research on social behavior, fruit flies are especially fruitful for such investigation owing to the abundance of tools that can facilitate all levels of biological analysis from genetics and

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neuroscience to behavioral and evolutionary biology (e.g. Ashburner 1989; Greenspan 2004; Zhang et al. 2010).

To enhance our knowledge of the evolutionary biology of sociability, we need further information about topics such as heritable variation in sociability, genetic correlations between life stages and sexes, and heritable variation in the plasticity of sociability. There are currently limited data regarding genetic variation in sociability and its mechanistic basis. By far, the most established research on the genetics of sociability involves mouse models of autism spectrum disorder. This line of research has identified a large variety of genes that influence social behavior (Moy and Nadler 2008; Silverman et al. 2010; Tuttle et al. 2017). Perhaps the best known case of natural genetic variation in sociability is the solitary and social forms of the nematode *Caenorhabditis elegans*. Solitary foragers disperse across a bacterial food substrate and feed alone, whereas social foragers aggregate and form clumps of up to several hundred individuals (De Bono and Bargmann 1998). In fruit flies, individuals from five distinct genetic lines varied in their social environmental choice (Saltz 2011), and work in our laboratory documented genetic variation in inter-individual distance among 29 distinct inbred lines (Anderson et al. 2016). Finally, in humans, personality traits associated with sociability including extraversion and the number of friends are highly heritable (Fowler et al. 2009; van den Berg et al. 2016). As noted earlier, sociability is also affected by the environment (Macdonald 1983; Johnson et al. 2002). We know, however, of no research assessing genetic variation in the plasticity of sociability.

We developed a new apparatus to critically address sociability, defined as the tendency to engage in non-aggressive activities with other individuals. A few laboratories, including ours, have used a variety of protocols to quantify social behavior in fruit flies (Tinette et al. 2004; Bolduc et al. 2010; Saltz 2011; Saltz and Foley 2011; Schneider et al. 2012; Lihoreau et al. 2016; Philippe et al. 2016; Anderson et al. 2017; Fernandez et al. 2017). Most notably, some protocols focused on social influences on food search behaviour (Tinette et al. 2004; Lihoreau et al. 2017). Other protocols measured inter-fly distance (Bolduc et al. 2010; Anderson et al. 2017; Fernandez et al. 2017). A few studies relied on social network analyses (Schneider et al. 2012; Pasquaretta et al. 2016). Finally, one study examined how male–male aggression influenced male and female fly distributions among food patches (Saltz and Foley 2011). While the other protocols are highly illuminating, they do not provide the critical feature that we wished to quantify, which was individuals' decisions to either join others or be alone at a food patch while controlling for food searching and sexual interactions and including in the analyses all individuals in each arena. Our new protocol allowed groups of same-sex flies from a given genetic background to arrange themselves according to their social preference inside arenas with distinct compartments separated by traversable barriers (Fig. 1).

We conducted a set of experiments addressing the following questions. First, what is the magnitude of genetic variation in sociability? Second, is there a genetic correlation in sociability between males and females? Third, are there key behavioral determinants, such as levels of activity, aggression, or non-aggressive interactions, that correlate with the observed genetic variation in sociability? Finally, do distinct genotypes respond differently to their social environment? That is, is there genetic variation in the plasticity of sociability?

Methods

General

We chose 60 Wolbachia-free lines from the *Drosophila* Genetic Reference Panel (DGRP). These lines were derived from mated females caught in Raleigh, North Carolina, USA, whose progeny were inbred through 20 generations of full-sibling mating (Mackay et al. 2012). We maintained these lines in vials with 5 mL of standard food medium (1L = 90 g sucrose, 75 g cornmeal, 10 g carrageenan, 32 g yeast, and 2 g methyl paraben dissolved in 20 mL ethanol) in an environment chamber at 25 °C, 50% relative humidity, and on a 12:12 light cycle with lights on at 10 AM.

In order to lessen the deleterious effects of inbreeding on the fruit fly nervous system that are observed in the majority of the inbred DGRP lines (Zwarts et al. 2015), we used

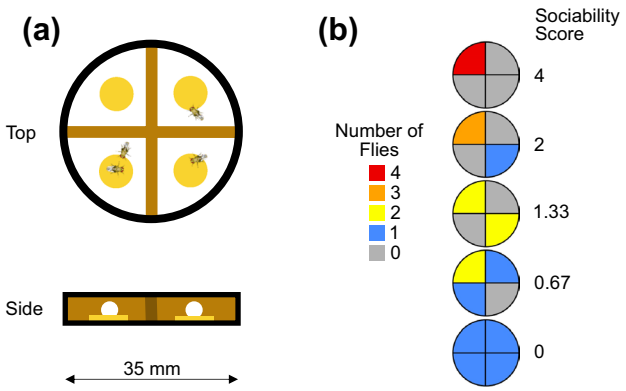

Fig. 1 **a** Diagram illustrating top and side views of the arenas used for quantifying sociability. Yellow circles (top) or rectangles (side) indicate standard food patch discs, and brown rectangles indicate barriers between quadrants. Holes allowing the flies to move between quadrants are visible in the side view. **b** Diagram illustrating some of the possible arrangements of flies in the sociability arenas and all of the possible values for the sociability score (calculated as variance/mean number of flies in each quadrant), with most to least social arrangements displayed from top to bottom. (Color figure online)

F1 hybrid flies (hereafter DGRP hybrids) generated from crosses between males from each of 59 DGRP lines and females from a randomly-chosen standard line, DGRP-83. For brevity, we refer to the DGRP hybrids based on their paternal line. We allowed virgin females of DGRP-83 to mate with males from each of the other 59 lines and lay eggs in food vials with 5 mL of standard food and a sprinkle of live yeast. We maintained consistent rearing densities by removing excess eggs from the vials. We collected experimental DGRP hybrid flies 11 days after egg laying. To avoid the deleterious effects of CO_2 anesthesia (e.g. Bartholomew et al. 2015), we sexed and transferred flies using gentle aspiration.

We analyzed the data with general linear mixed-effects models in R version 3.3.3 (R-Core-Team 2014) with the package lme4 version 1.1-12 (Bates et al. 2014). For tests of fixed effects, we report Wald χ^2 values generated with the Anova function from the car package version 2.1-4 (Fox and Weisberg 2011). For random effects, we report p-values calculated as the fraction of parametric bootstrapped likelihood ratio test (LRT) statistics (with 10,000 iterations) that were larger than the observed LRT values, using the package pbkrtest version 0.4-7 (Halekoh and Højsgaard 2014). To generate 95% confidence intervals on model variance components and heritability estimates, we performed hierarchical non-parametric bootstrapping (with 10,000 iterations). In each iteration, sampling with replacement occurred first at the level of DGRP hybrids, and then samples within DGRP hybrids. This approach also enabled us to verify model estimates for general linear mixed-effects models since the assumption of normally distributed residuals of these models was violated, due to our measure of sociability being bounded. We used custom code for the bootstrapping based on Roles et al. (2016). For tests of significance of correlations between traits, we report results from Spearman's rank correlations, and bootstrapped 95% confidence intervals (with 10,000 iterations) generated with the boot package (Canty and Ripley 2017). We describe further statistical details in the sections below.

Genetic variation in sociability and correlation between the sexes

Quantifying genetic variation in sociability

We collected DGRP hybrid adults from each of the 59 crosses within 8 h of eclosion, and transferred a mixed sex group consisting of five males and five females from the same cross into each vial containing 5 mL of standard food. We left the flies in an environment chamber for 3 days to gain social experience. Approximately 72 h post-eclosion, at 9:00 AM, we transferred groups of 4 same-sex flies from the same vial into each test arena. The test arenas (Fig. 1a) were circular petri dishes (35 mm diameter × 10 mm high) with wooden partitions that divided the dish space into four quadrants. Each quadrant had a single food patch (5 mm diameter × 1 mm thick) with a layer of grapefruit/yeast solution (3 g yeast per 100 mL grapefruit juice) on the surface of the food. Flies could move between quadrants through 3 mm holes in the center of each partition. Our preliminary experiments indicated that flies frequently travelled between quadrants.

We aspirated live flies into the arenas through a 3 mm hole in the dish lid, such that the starting arrangement consisted of one fly in each quadrant. We placed the dishes into four large semi-transparent plastic containers with opaque lids (l × w × h:51 × 31 × 30 cm), which were humidified at ~75% RH. We left the flies to explore the arenas and acclimatize from 11:30 AM to 2:30 PM. Then every 10 min from 2:30 PM to 4:00 PM, an observer blind to DGRP hybrid identity recorded the number of flies in each quadrant of each dish through a thin opening in the box lids. We tested each group of flies only once. We counterbalanced which box the crosses were tested in, and the location within each box across days. Our sample sizes ranged from 10 to 14 arenas per each DGRP hybrid and sex.

We quantified the sociability score of each group of four flies in each arena at each time point using the aggregation index. The aggregation index is a standard ecological measure (Krebs 1999), which we have previously used successfully to quantify social behaviour in fruit fly larvae (Durisko et al. 2014), and is calculated as the variance divided by the mean number of flies in each quadrant. In our protocol, sociability scores could take five possible values ranging from 0 to 4 (Fig. 1b), with 0 representing the least sociable distribution (one fly per quadrant), and 4 representing the most sociable distribution (all flies in the same quadrant). A value of 1 (mean = variance) represents random distribution, which could only be taken on by averaged observations. We pooled the data over the 1.5 h observation period as there was very little among-DGRP hybrid variation for the temporal effects compared to the overall among-DGRP hybrid effects (about 1/1000th the variation). We constructed a general linear mixed model with pooled sociability scores as the dependent measure, day and box as simple random effects, and sex as both a fixed effect and allowed to vary by the random effect of DGRP hybrid. We constructed reduced models to test each of the simple random effects, and models that reduced DGRP hybrid to a simple random effect or omitted it to test for a genotype by sex interaction and main effect of genotype, respectively. We also constructed full models separately for each sex with day, box and DGRP hybrid as simple random effects, and reduced models to test for the sex-specific effects of DGRP hybrid, day, and box. We tested for significant correlations between male and female sociability using sex-specific means of each DGRP

hybrid, and also using model-generated sex-specific best linear unbiased predictors of the random effects of each DGRP hybrid. Because the correlations of the best linear unbiased predictors were very similar to the correlations of the means of each DGRP hybrid, we only report the correlations of the latter. We used non-parametric bootstrapping to generate estimates and confidence intervals of sex-specific broad-sense heritabilities (H^2) of sociability. H^2 was estimated as $V_G/(V_G + V_E) = 2\sigma_l^2/(2\sigma_l^2 + \sigma_e^2)$, where V_G is genetic variance, V_E is environmental variance, σ_l^2 is the among-DGRP hybrid variance component, and σ_e^2 is the error variance (including both the residual and day variance components) (Falconer and Mackay 1996; Shorter et al. 2015). We multiplied the among-DGRP hybrid variance component by 2 to account for the shared maternal line of the DGRP hybrids. We also calculated sex-specific values of the coefficient of genetic variation (CV_G), which is a scaled measure of genetic variation that is not environment-specific, and therefore more easily compared to other traits (Houle 1992). We calculated CV_G as $\sqrt{V_G}/\overline{X} = \sqrt{2\sigma_l^2}/\overline{X}$, where $\overline{X}$ is the sex-specific overall mean sociability score. Note that the DGRP hybrid males all received an X chromosome from the same standard maternal line, DGRP-83. This means that our measures of genetic variation included all chromosomes in the hybrid females but only the autosomal chromosomes in the hybrid males.

Follow-up sociability experiment in a subset of 16 DGRP hybrids

Our initial analyses revealed a weak genetic correlation in sociability between males and females (Fig. 2c). In order to better characterize the genetic correlation between the sexes, we repeated the sociability test on a subset of 16 DGRP hybrids. We used a randomness generator to choose four DGRP hybrids from each quartile of the mean sociability scores in males from the 59-DGRP hybrid assay. The bars of these 16 hybrids are marked with white dots in Fig. 2a. We based this choice on the male data due to the larger genetic variation in males compared to females (Fig. 2a, b). The methods for rearing the hybrids, housing, and testing were similar to the methods for the 59-DGRP hybrid assay above. We assayed a total of 10 arenas per each DGRP hybrid and sex. We analyzed the data using general linear mixed models, and tested for significant male–female correlations as in the assay using the 59 DGRP hybrids.

Behavioural determinants of sociability

To gain insight into the mechanisms that generate the observed genetic variation in sociability score, we conducted two experiments. First, we quantified the activity level of individual flies to assess whether genetic variation in activity is correlated with sociability scores. Second, we video recorded a sub-sample of DGRP hybrids in the sociability test arenas (Fig. 1) and conducted detailed behavioral analyses of key factors that we expected to influence the sociability scores. These included (i) another measure of activity, the frequency of movement between quadrants, (ii) aggression frequency, and (iii) non-aggressive encounter frequency.

Genetic variation in activity

We assayed 57 of the 59 DGRP hybrids used in the sociability assay for baseline individual activity. Two of the paternal lines, DGRP-757 and DGRP-158, died out between the two experiments. We used the same protocol for rearing, collecting and housing the hybrids that we used for the sociability assay. Approximately 72 h post-eclosion, at 9:00 AM, we aspirated a single fly from each mixed-sex housing vial, either male or female depending on the day, into a small snap-cap vial (22 mm diameter × 48 mm long). The lids of the snap cap vials had a small pinhole for ventilation. Each snap-cap vial had a single food patch (5 mm diameter × 1 mm thick) with a layer of grapefruit/yeast solution. We placed the vials into 1 of 2 Drosophila activity monitors (Trikinetics Inc.; software version 3.08). We placed each monitor in an upright position in 1 of 2 opaque plastic containers (l × w × h:51.5 × 36 × 41 cm) that were humidified at ~75% RH. The vials were held in the monitor slots in a horizontal position, with ~7 mm of clearance between the infrared sensors and the surface of the food patch. We placed an LED lightbulb over a hole in the center of each container lid to illuminate the monitors from above. We left the flies to acclimatize from 11:30 AM to 2:30 PM. From 2:30 PM to 4:00 PM, activity was automatically recorded as the total number of times that each fly crossed the ring of infrared sensors that surrounded each snap-cap vial during the 90 min test period. We assayed one fly from each DGRP hybrid cross per day, alternating testing males and females daily, over 30 days. We counterbalanced which monitor and which position within each monitor the DGRP hybrids were tested in across days. In total, we assayed between 10 and 15 replicates per DGRP hybrid and sex.

We analyzed the data by constructing zero-inflated negative binomial generalized linear mixed models using the package glmmTMB version 0.1.1 (Brooks et al. 2017) because a high proportion of flies (21%) had activity scores of 0. For the conditional model, we included the number of times the fly crossed the infrared sensor as the dependent measure, activity monitor as a fixed effect, day as a simple random effect, and sex as both a fixed effect and varied by the random effect of DGRP hybrid. For the zero-inflation model, we included sex and activity monitor as fixed effects and DGRP hybrid as a simple random effect. We tested

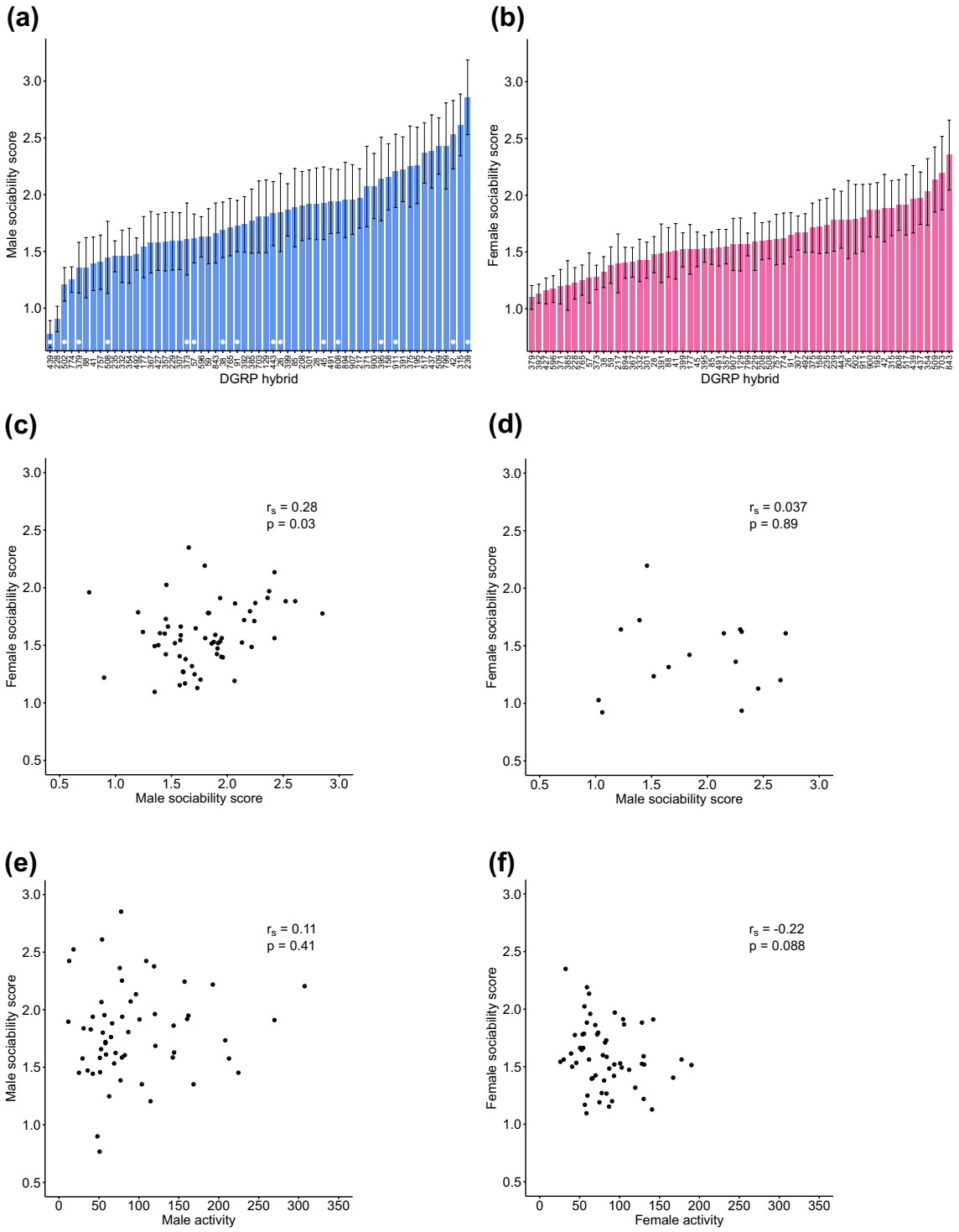

Fig. 2 Genetic variation in sociability and correlations in sociability across sexes and between sociability and activity. Mean sociability scores ± 1 standard error of the mean (SEM) of 59 DGRP hybrids are shown in **a** males and **b** females. Bars are ordered along the x axis by increasing mean, and are labeled according to the paternal DGRP line. The bars of the 16 DGRP hybrids used in the replicate sociability assay are marked with white dots in Fig. 2a. Genetic correlations between males and females for sociability are shown in **c** the original 59 DGRP hybrid assay and **d** the 16 DGRP hybrid subset assay. Correlations between sociability and activity are shown in **e** males and **f** females. Points in the scatterplots represent means for each DGRP hybrid generated from the raw data

for significant correlations between sociability and activity means of each DGRP hybrid, and between sociability and the model-generated best linear unbiased predictors of the random effects of each DGRP hybrid for activity (from both the conditional and zero-inflation models). We found the correlations of the best linear unbiased predictors to be close to the correlations of means of each DGRP hybrid, so we only report the latter.

Inter-quadrant movement frequency, aggression, and non-aggressive encounters

We conducted video recording during the replicate sociability assay with the 16 DGRP hybrids described above. We focused on males from 8 of the 16 DGRP hybrids, with 2 randomly chosen from each quartile. After introducing the flies into the test arenas, we video recorded them for 1 h using 6th generation Apple iPod Touch devices at 30 frames per second. We focused on the first hour because we assumed that the initial interactions in the arena would be the most important in establishing fly distributions in the arenas and hence their sociability scores. Overall, we video recorded two male arenas from each of the eight selected DGRP hybrids each day for 5 days, for a total of ten video observations per DGRP hybrid.

Observers blind to DGRP hybrid identity recorded aggressive interactions from minutes 5–20, and non-aggressive interactions and boundary crossing from minutes 0–60 of each video using BORIS behaviour coding software version 3.50 (Friard and Gamba 2016). Observers recorded aggressive interactions, which included lunging, wing threat, high-level fencing, charging, holding, boxing and tussling (Chen et al. 2002; Baxter and Dukas 2017). Because almost all aggressive events were lunges, we quantified aggression as the lunging frequency. Observers recorded non-aggressive encounters using the same criteria that we established in a previous experiment (Anderson et al. 2017), in which we defined these encounters as inspections of one fly by another (e.g. licking or prodding with legs), or the movement of one fly towards another followed by a response from the other fly (e.g. wing fluttering or moving away). Observers recorded boundary crossings as a fly moving from one quadrant to another. We analyzed the data using general linear mixed models as in the 59 DGRP hybrid and replicate sociability assays and included inter-quadrat movement rate, lunging rate, and non-aggressive encounter rate as quantitative predictors.

Genetic variation in the plasticity of sociability

We assayed sociability in males of 16 DGRP hybrids across 4 pre-test social environments. We used the same DGRP hybrids as those in the replicate sociability assay, except for the hybrid with paternal line DGRP-38, which died out between experiments. We replaced this line with a hybrid with paternal line DGRP-843, which we randomly selected from the same quartile as DGRP-38. After sexing the flies, we introduced males of each DGRP hybrid cross into standard food vials with 1 of 4 social environments for the 3-day pre-test period: males housed individually, single males housed with single females, males housed in groups of 4, and mixed sex groups of 4 males and 4 females. Having males with and without females allowed us to test both a natural situation (mixed sex groups) and a situation that controls for male mating status (male only groups). On the morning of the test day, when all flies were about 72 h post eclosion, we transferred males from the same social treatment and DGRP hybrid cross to the test arenas. For the treatments with four males in a vial, we transferred groups that were housed together into the same arena. Our sample sizes were either 9 or 10 arenas per each DGRP hybrid and treatment.

We analyzed the data by constructing general linear mixed models as in the other sociability assays, with pooled sociability scores as the dependent measure, number of males (1 vs. 4) and female presence (yes vs. no) as fixed effects, and with both effects allowed to vary by the random effect of DGRP hybrid (equivalent to random slopes models). We initially included both day and box as simple random effects, but removed them as the variance estimates were very close to zero. We used non-parametric bootstrapping to generate estimates and confidence intervals of the broad-sense heritabilities (H^2) of the plasticity of sociability under the different social environment contexts. H^2 was estimated as $V_G/(V_G + V_E) = 2\sigma^2_{l\times t}/(2\sigma^2_{l\times t} + \sigma^2_e)$, where V_G is genetic variance, V_E is environmental variance, $2\sigma^2_{l\times t}$ is the DGRP hybrid-by-treatment interaction variance component (treatment being number of males or female presence), and σ^2_e is the error variance (Scheiner and Lyman 1989). We also calculated coefficients of genetic variance (CV_G) estimates as $\sqrt{V_G}/\overline{X} = \sqrt{2\sigma^2_{l\times t}}/\overline{X}$, where $\overline{X}$ is the overall mean sociability score.

Results

Genetic variation in sociability and correlation between the sexes

We found significant genetic variation in sociability among the 59 DGRP hybrids in both males (range of mean sociability scores: 0.77–2.85; $p < 0.001$, Fig. 2a) and females (range of mean sociability scores: 1.10–2.35; $p < 0.01$, Fig. 2b). The broad-sense heritability of sociability was 0.24 (95% CI [0.14, 0.35]) for males, and 0.21 (95% CI [0.11, 0.31]) for females. The estimated coefficients of genetic variance

(CV_G) were 0.31 (95% CI [0.22, 0.39]) for males and 0.24 (95% CI [0.16, 0.31]) for females. On average, males were more sociable than females (1.81 vs. 1.60 mean sociability scores respectively; Wald $\chi^2_1 = 13.16$, $p < 0.001$) but there was a significant DGRP hybrid-by-sex interaction ($p < 0.01$). Within the male data, there was no significant effect of day ($p = 0.27$) or observation box ($p \approx 1$); within the female data, there was a significant effect of day ($p < 0.01$) but not of observation box ($p = 0.09$).

In the analysis of the 59 DGRP hybrids, we found a weak significant positive genetic correlation between the sexes for sociability ($r_s(57) = 0.28$, $p = 0.03$, 95% CI [0.01, 0.51]; Fig. 2c). However, in the follow up experiment using a subset of 16 DGRP hybrids, we found no correlation in sociability scores between the sexes ($r_s(14) = 0.037$, $p = 0.89$; 95% CI [−0.55, 0.63]; Fig. 2d).

Behavioural determinants of sociability

Genetic variation in activity

We found no significant genetic correlations between activity and sociability in either males ($r_s(55) = 0.11$, $p = 0.41$, 95% CI [−0.17, 0.37]) or females ($r_s(55) = -0.22$, $p = 0.088$, 95% CI [−0.44, 0.02]; Fig. 2e, f).

Inter-quadrant movement frequency, aggression, and non-aggressive encounters

We found no significant effects of inter-quadrant movement rate (Wald $\chi^2_1 = 0.035$, $p = 0.85$), lunging rate (Wald $\chi^2_1 = 0.72$, $p = 0.40$) and non-aggressive encounter rate (Wald $\chi^2_1 = 0.31$, $p = 0.58$) during the initial acclimatization period on subsequent sociability in males of eight DGRP hybrids. We noted that means of non-aggressive encounter rates were correlated with means of lunging frequencies ($r_s(6) = 0.85$, $p = 0.008$). However, taking either encounter or lunging frequency out of the model did not change the effects of the other quantitative predictors. We also noted that 6 of 8 DGRP hybrids had mean lunging rates close to 0 (between 0.1 and 0.9 lunges per 15 min), DGRP hybrid-26, which had a mid-level mean sociability score in the subset assay, had a mean lunging rate of 4.1 per 15 min, and DGRP hybrid-502, which had the lowest mean sociability score among the eight video-recorded hybrids, had the highest mean lunging rate (21.3 per 15 min).

Genetic variation in the plasticity of sociability

We found a significant effect of the number of males housed together during the pre-trial period on subsequent male sociability, with males housed with other males being more sociable than males housed singly (Wald $\chi^2_1 = 37.52$, $p < 0.001$; Fig. 3a). However, female presence had no significant effect on subsequent male sociability (Wald $\chi^2_1 = 1.55$, $p = 0.21$; Fig. 3a). There was significant genetic variation in sociability among males of the 16 DGRP hybrids ($p < 0.001$; Fig. 3c). The interaction between DGRP hybrid and the number of males housed together approached significance ($p = 0.083$; Fig. 3b), and the interaction between DGRP hybrid and female presence was significant ($p = 0.038$, Fig. 3c). The broad-sense heritability of the plasticity of sociability was 0.22 (95% CI [0.04, 0.41]) in the context of number of males housed together, and 0.26 (95% CI [0.07,

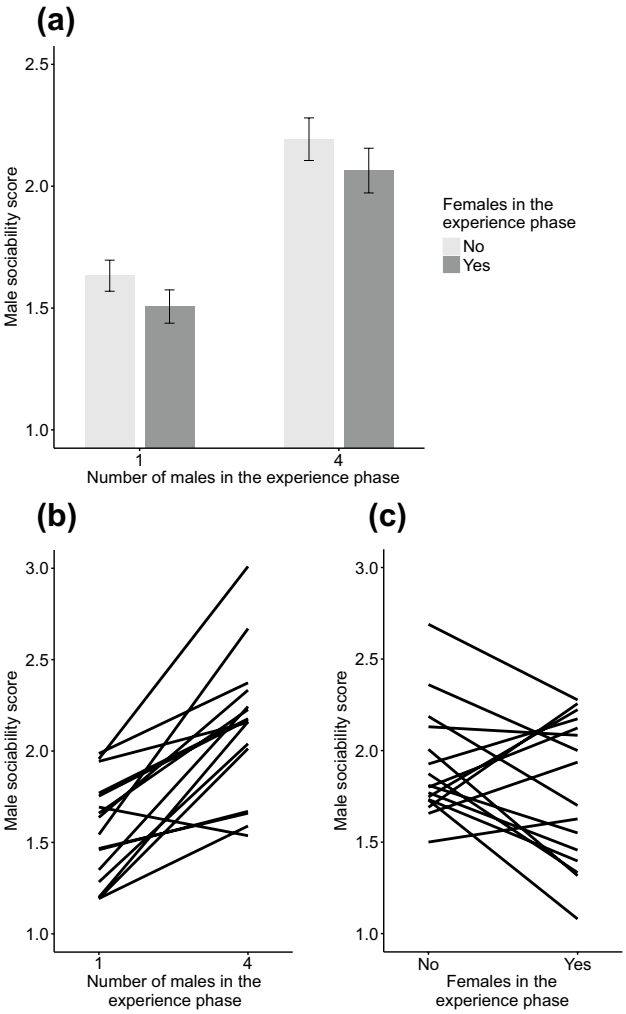

Fig. 3 Social plasticity in males of 16 DGRP hybrids. All tests involved calculating the sociability scores of groups of 4 males after they had experienced distinct social settings. **a** Shows the mean (±1 SEM) sociability scores averaged across the 16 DGRP hybrids for males previously housed singly with no females, singly with a female, in groups of 4 with no females, and in groups of 4 males + 4 females. **b, c** Show the mean sociability scores for each of the 16 DGRP hybrids (reaction norm lines) as a function of their previous social experience, **b** alone or in groups of 4 males in the experience phase, and **c** without or with females in the experience phase. Error bars in **b** and **c** are omitted for clarity

0.45]) in the context of female presence. The coefficients of genetic variation (CV_G) of the plasticity of sociability were 0.26 (95% CI [0.10, 0.41]) in the context of number of males housed together, and 0.30 (95% CI [0.14, 0.44]) in the context of female presence.

Discussion

Our major findings were, first, that there was significant genetic variation in sociability in both males and females with broad-sense heritability of 0.24 and 0.21 respectively (Fig. 2a, b). Second, there was little genetic correlation in sociability between the sexes (Fig. 2c, d). Third, sociability scores were not correlated with activity levels (Fig. 2e, f), aggression, or non-aggressive inter-individual interactions. Finally, we found genetic variation in social plasticity among the DGRP hybrids (Fig. 3). We discuss these results in turn.

We defined sociability as the tendency to engage in non-aggressive activities with other individuals and developed a new apparatus to quantify it. In that apparatus, each of four individual flies decided whether to join others, stay with others, deter others from joining, or move to an unoccupied food patch (Fig. 1a). A glance at Fig. 2a, b indicates first, that flies clearly did not avoid each other as only two DGRP hybrids had a sociability score below 1 (see Fig. 1b). Second, most hybrids had a sociability score above the random value of 1. Finally, no hybrids approached the maximum score of 4. Hence we can conclude that fruit flies are moderately sociable. We have reached similar conclusions in two previous studies using distinct fly life stages, lines and protocols. The first project involved larvae that were descendants of wild-caught fruit flies (Durisko et al. 2014) and the other project included larvae and adults of 29 inbred DGRP lines (Anderson et al. 2016). Interestingly, our sociability apparatus is conceptually similar to the two-tube version of the tube co-occupancy test, which was recently developed for quantifying sociability in mice (Figs S2A and 1E in Tuttle et al. 2017). The tube co-occupancy test is supposed to advance research on mouse sociability as it allows for the more realistic direct contact between individuals. This does not occur in the traditional apparatuses, which rely on testing the proximity of a focal mouse to either a mouse or control object placed beyond screens (Tuttle et al. 2017).

As noted in the introduction, there is currently limited information on natural genetic variation in sociability (De Bono and Bargmann 1998; Saltz 2011; Anderson et al. 2016; Ward and Webster 2016). In humans and other mammals, much of the research effort has focused on candidate genes for autism (Abrahams and Geschwind 2008; Moy and Nadler 2008) and for pair bonding (Donaldson and Young 2008; Walum et al. 2008). In humans, social skills are highly heritable (Viken et al. 1994; Scourfield et al. 1999; Rettew et al. 2008; van den Berg et al. 2016) and variation in a few genes has been linked to measures related to sociability (Skuse et al. 2014; Pearce et al. 2017). Twin studies in humans have provided some estimates of the heritability of social behaviours, such as altruism (Rushton et al. 1986), antisocial behaviour (Mason and Frick 1994) and reciprocal social behaviour (Constantino and Todd 2000), and all have been found to be highly heritable. While we found significant genetic variation in sociability among the 59 DGRP hybrids, we cannot yet link that variation to either survival or reproduction. Similarly, we will require further work for linking the variation in sociability among the DGRP hybrids to specific genes and neurobiological pathways. Our estimate of the heritability of sociability (0.24 for males and 0.21 for females) is close to the typical estimate of the heritability of social behaviours, which is around 0.3 (Stirling et al. 2002).

Our data indicated mostly independent regulation of sociability in males and females, in that there was little evidence for a genetic correlation. The most likely explanation for this is that sociability is determined by mechanisms similar to the ones regulating sex specific traits related to maximizing mating opportunities in males and egg laying in females. Interestingly, males' sociability scores were significantly higher than females' (Fig. 2a, b) but there was significant DGRP hybrid-by-sex interaction. We still cannot explain this pattern. While we are not aware of data pertaining to genetic correlations in sociability between the sexes, there are some relevant data on aggression. In fruit flies, artificial selection on male–male aggression resulted in a single line in which males were hyperaggressive but there was no change in female–female aggression (Penn et al. 2010). Mouse studies on male–female correlation in aggression are inconclusive, with some studies showing no correlation and others reporting positive correlation between male–male aggression and maternal aggression (Sandnabba 1996; Gammie et al. 2003). Finally, white throated sparrows (*Zonotrichia albicollis*) have two morphs, which are determined by an inversion polymorphism on chromosome 2. Both sexes of the white-striped morph show higher levels of some types of aggression than males and females of the tan-striped morph (Thorneycroft 1966, 1975; Thomas et al. 2008; Horton et al. 2014).

We conducted two assays to quantify behavioral correlates of sociability. First, we wished to verify that our sociability scores did not merely reflect genetic variation in levels of activity. For example, if docile flies just stayed where we placed them one per quadrant, we would have classified them as non-sociable (Fig. 1). We quantified the activity levels of individual flies so that our measures were not influenced by social interactions. While we found large genetic variation among the DGRP hybrids, it was not correlated with sociability (Fig. 2e, f). Our results are consistent with previous analyses using distinct protocols, which showed decoupling

of social behavior and activity in larval and adult fruit flies (Anderson et al. 2016). Similarly, measures of activity were not correlated with aggressive behavior in fruit flies (Rohde et al. 2017). Finally, activity and both male–male and female maternal aggression were not genetically correlated in mice (Gammie et al. 2003).

The second assay examining behavioral correlates of sociability involved scoring key behaviors from videos taken during the settlement of flies in the sociability arenas. As expected, our alternate measure of activity, the frequency of inter-quadrant crossing was not correlated with sociability. We found, however, no correlation between sociability and either aggressive or non-aggressive interactions. Superficially, one might expect a negative correlation between sociability and aggression. Mechanistically, what we found was next to no aggression in 6 of the 8 DGRP hybrids examined, suggesting that overt aggression was not the driving force behind the genetic variation in sociability. Ultimately, one might expect a complex interaction between sociability and aggression. The simple reason for this is that the payoff from aggression may be higher in social groups than among solitary individuals. For example, being the dominant member of a social group can provide one with preferential access to resources such as food, shelter and mates. Indeed a phylogenetic analysis of mammals indicated much higher levels of lethal aggression in social than in solitary species (Fig. 2 in Gómez et al. 2016). Research on humans also indicated no correlation between aggression and sociability (Buss and Perry 1992). While there is no theoretical foundation for predicting an association between sociability and non-aggressive interactions, our previous work indicated a positive correlation between non-aggressive interactions and inter-individual distance. That is, the lines where individuals were physically closest together had the fewest interactions (Anderson et al. 2017). We intend to quantify the association between our current sociability index, nearest neighbor index and fly interactions in future work.

Finally, in the experiment on social plasticity, we quantified genetic variation in males' sociability in response to two relevant factors, social isolation and exposure to females. Overall, we found significant social plasticity, with males housed in groups being more sociable than males held alone prior to the test (Fig. 3a). Being housed with or without females, however, did not significantly affect male sociability (Fig. 3a). Our former results are consistent with Simon et al. (2012), who found that social isolation subsequently led to greater inter-fly distance. Studies on fruit fly aggression are also consistent with the conclusion that flies held in isolation are subsequently less sociable than flies held in group as indicated in higher levels of aggression (Hoffmann 1990; Ueda and Kidokoro 2002; Wang et al. 2008). Similar results of isolation increasing subsequent aggression are known in many other species (Allee 1942; Valzelli 1973).

While the effects of social isolation on sociability are somewhat established, the effects of prior experience with females are not as clear. Unlike us, Simon et al. (2012) reported shorter inter-fly distance in males previously housed with females than in males kept only with males. Simon et al. (2012) measured inter-fly distance in a large arena with 40 flies and no food, so our protocols are rather distinct. The effects of prior experience with females on aggression are similarly conflicting. Yuan et al. (2014) found that males previously housed with females were less aggressive than virgin males. In two experiments using distinct protocols, however, we found no difference in aggression based on prior sexual experience (Baxter and Dukas 2017). A possible explanation for the different results is genetic variation in social plasticity among the lines used in the different studies.

We found genetic variation in social plasticity, which was marginally significant when we placed males either alone or with three other males (Fig. 3b), and significant when we housed males with or without females prior to the test (Fig. 3c). Most notably, about half the DGRP hybrids had higher sociability scores after being held with than without females, while the other half showed the opposite pattern. We will require further experiments to elucidate the social dynamics during the experience phase that generate the distinct patterns of social plasticity. We will also need additional work to find out the mechanisms underlying social plasticity. The most relevant study on genetic variation in social plasticity compared aggression in males kept in mixed sex groups and in isolated males of 87 inbred fruit fly lines. That study documented significant genotype by social environment interaction (Rohde et al. 2017). Unexpectedly though, many of the lines showed greater aggression after housing in groups than alone (Fig. 2 in Rohde et al. 2017), which is inconsistent with the well replicated, robust effects of social isolation on aggression discussed above (Hoffmann 1990; Ueda and Kidokoro 2002; Wang et al. 2008). In humans, natural variation in the gene encoding the neurotransmitter-metabolizing enzyme, monoamine oxidase A (MAOA), has been linked to plasticity in aggression, with only carriers of the low activity allele responding to maltreatment with heightened aggression (Caspi et al. 2002; Gallardo-Pujol et al. 2013). There are few other estimates of the genetic variation in social plasticity in particular or behavioral plasticity in general because estimating variation in the slopes of behavioral reaction norms can be challenging (Araya-Ajoy and Dingemanse 2017). In the three-spined stickleback (*Gasterosteus aculeatus*), there was limited evidence for population-specific genetic variation in plasticity of a few animal personality traits including sociability in the context of predation risk (Dingemanse et al. 2009) and significant genetic variation in plasticity of exploration behaviour in novel environments (Dingemanse et al. 2012). In a

recent study, the heritability of the plasticity of aggression in wild great tits (*Parus major*) was estimated to be 0.266, but this estimate was highly uncertain (Araya-Ajoy and Dingemanse 2017). Also in great tits, the heritability of the plasticity of egg-laying date was estimated as 0.3 (Nussey et al. 2005). Our estimate of the heritability of social plasticity in fruit flies (0.21–0.24) was similar to these estimates.

In sum, we documented large genetic variation in sociability and some genetic variation in social plasticity in fruit flies. These finding open up exciting opportunities for future work on the mechanisms that underlie that variation as well as the ecological and evolutionary forces that maintain it.

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Compliance with ethical standards

Conflict of interest AM Scott, I Dworkin and R Dukas declare that they have no conflict of interest.

Human and animal rights and informed consent This article does not contain any studies with human participants or animals performed by any of the authors.

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